韓 國 菌 學 會 誌 第10卷 第1號
Kor. J. Mycol. Vol. 10, No. 1, 7~13, 1982

Morphological and Cultural Characters of *Didymella bryoniae* on Seeds and Culture Media

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種子 및 培地上에서의 오이類 덩굴마름병균의 形態的 및 培養的 特徵

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Abstract : Habit characteristics of imperfect and perfect stage of *D. bryoniae* encountered on naturally infected seeds of cucumber and pumpkin were studied by the blotter method and compared with those grown on Difco potato dextrose agar (PDA), V-8 juice agar and water agar leaf medium (WALM).

Most of the pycnidiospores obtained from each isolate of this fungus grown on PDA were non-septate and microtype. Non-septate pycnidiospores were predominanted in all isolates, but a macrotype of the non-septate and a number of uniseptate pycnidiospores were produced on V-8 juice agar and water agar leaf medium.

On seed the pycnidiospores were mostly non-septate, but rarely uniseptate ones were also found. On radicle of cucumber seed, the pycnidiospores were non-septate and uniseptate but small percentage biseptate with somewhat constricted at septa. Pycnidiospores produced on V-8 juice agar and water agar leaf medium were similar to those produced on seeds.

In the present investigation the perithecia were mostly globose to subglobose with apical papillate ostiole and whitish spore masses formed on the ostiole of perithecia, either on naturally infected seed or on culture media. The mature perithecia were dark brown to black. They were partially embedded or erumpent on seed coat and culture media. The perithecia varied in size within a much narrower range than the pycnidia. But perithecial formation of this fungus on PDA, V-8 juice agar, WALM and seed varied considerably depending upon isolate and culture media.

Introduction

The gummy stem blight of cucurbits incited by *Didymella bryoniae*(Auersw.) Rehm is one of the most important diseases of the cucurbits in many countries (Cruger & Schneider 1964; Fletcher & Preece 1966; Kagiwata 1967; Proctor & Young 1967; Schenck 1968; Schenck 1968; Figueiredo *et al* 1970). It has a wide host range in cucurbits including cucumber(*Cucumis sativus* L.), muskmelon(*Cucumis melo* L.), oriental melon(*Cucumis melo* var. *makuwa* Makino), pumpkin(*Cucurbita peop* L.) and watermelon (*Citrullus vulgaris* Shrad.) and shows a variety of symptoms which have been referred to as leaf-spot, stem canker, vine wilt and black fruit rot.

Although Fautrey and Roumeguere recognized the cucurbit black rot fungus on a variety of chinese cucumber to be a species of *Ascochyta* and described it under the name *Ascochyta cucumis* in France as

early as 1891, the pleomorphism and variability of this fungus were the cause for some of the confusion in its early classification of the imperfect stage. The small continous micropycnidiospores which predominate in nature on the host, apparently led Chester (1891) to describe the fungus as *Phyllosticta citrullina* on watermelon and led Keissler(1923) to report *Phyllosticta orbicularia* Ell et Ev. on *Cucumis melo* L.

Habit characteristics of imperfect and perfect stage of *Didymella bryoniae* encountered on naturally infected seeds of cucumber and pumpkin were studied by the blotter method and compared with those grown on Difco PDA, V-8 juice agar and water agar leaf medium.

Materials and Methods

The pathogen was isolated from cucurbit seeds in petri-dish cultures by spore dilution method from exuded conidia. Transfers were then made from the margin of young colonies to agar slants. For comparative study of the growth and morphological characters, pure cultures of different isolates of *D. bryoniae* were grown on Difco PDA and V-8 juice agar (Miller 1955). The growth of *D. bryoniae* observed on the naturally infected seeds of cucumber and pumpkin was compared with those grown on water agar leaf medium(Srinivasan *et al* 1971). Incubation temperature used in this study was at 20°C and near untraviolet light(NUV) was provided 12 hours each day for 7 days.

Observations on the type of fruiting structures developed, were made under stereoscopic microscope. The morphology of pycnidiospores shape, size, septation, etc.was studied under a compound microscope. For each isolates, the average length, width and as well as the range, were determined, by measuring 30 fruiting structures.

Results

Cultural characters of the four isolates of *D. bryoniae* on PDA are described as Table I. On PDA the colony diameter increased by approximately 1cm per day at 20°C. On this medium the mycelium at first was white,but after two to three days it began to impart a colour to the agar from the center. Depending upon the isolates, the colour ranged from olive-green to dark olive to black or salmon to brown and usually concentric zones were present(Fig. 1). In cucumber isolates, the amount of aerial mycelium was abundant, white, becoming gray with age, but generally in pumpkin isolates aerial mycelium was almost absent, or sparsely scattered over the surface.

On V-8 juice agar aerial mycelium produced was less than on PDA and the submerged mycelium was rather thin and light coloured. On WALM fungus growth took place on the leaf substrate in the form of thin creeping mycelia growing into the substrate.

Table I. Cultural characters of four isolates of *Didymella bryoniae* from cucumber and pumpkin seeds on PDA seven days after inoculation.

Isolate no.	Types of mycelial growth and sporulating structures			
	Aerial mycelium	Submerged mycelium	Pycnidia	Perithecia
1 (cucumber)	vigorous, white becoming gray with age	dark olive to black	sporulation sparse	black bodies(perhaps protoperithecia) numerous
2 (cucumber)	vigorous, white becoming gray with age	dark olive to black	sporulation sparse	sporulation light
6 (pumpkin)	usually absent, very scanty when present	hyaline at first, becoming salmon to brown	sporulation heavy	no sporulation
8 (pumpkin)	usually absent, very scanty when present	hyaline at first, brown later	sporulation heavy	no sporulation

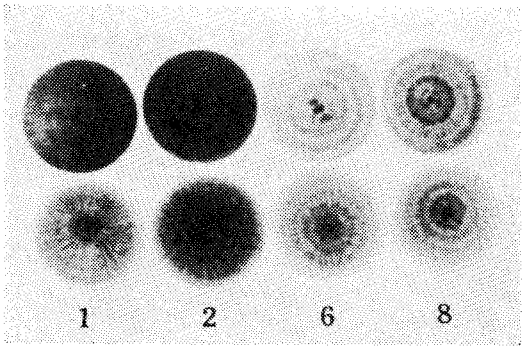

Fig. 1. Isolates of *Didymella bryoniae* grown on PDA(upper) and V-8 juice agar(below): from left to right, Isolate No. 1, 2, 6, 8.

On seed aerial mycelium usually absent, very scanty when present.

Fructification of each isolate on PDA and V-8 juice agar varied considerably depending upon isolate and substrate. All isolates produced pycnidia on both media, but isolate obtained from pumpkin seeds produced pycnidia considerably more than those from cucumber seeds on both media. On PDA and V-8 juice agar, pycnidia often lacked a definite shape and contained more than one chamber. These pycnidia were often multiostiolate. Spores exuded from pycnidia piled up on the surface of the agar, giving the cultures a granular appearance.

Isolates obtained from cucumber seeds produced perithecia on both media, but the isolates from pumpkin produced perithecia on V-8 juice agar only, sparsely. Perithecia were dark and somewhat smaller than the pycnidia, slower to develop and appeared first in the older, central portion of the plate. Whitish masses of exuded ascospores were formed on the ostiole of perithecia(Fig. 7). Perithecia were similar in shape and structure to those produced on cucurbit seeds.

On the WALM pycnidia and perithecia began to form within three days from the inoculated part of the surface of the leaf pieces, and extending to the agar substratum. The sporulating structures on WALM compared with on the naturally infected seeds are given Table Ⅱ. It was noticed that the appearance of pycnidia and perithecia formed on this medium closely resembled those observed on seeds in

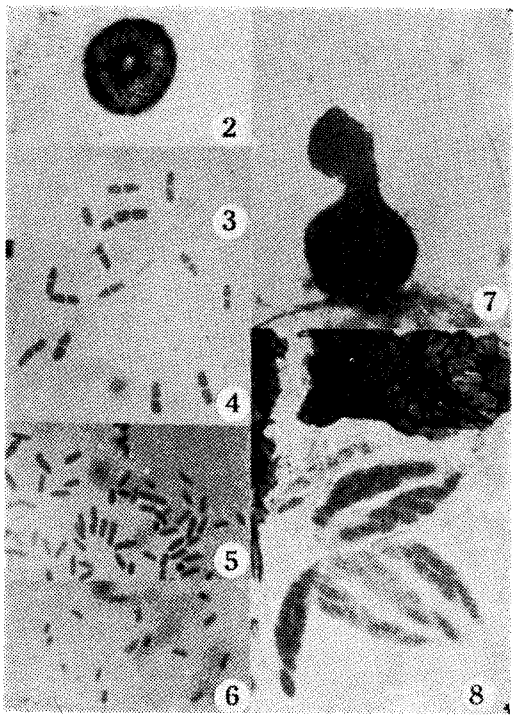

Fig. 2-8. Photomicrographs showing asexual and sexual stage of *Didymella bryoniae.*
2. Pycnidium, 3. Biseptate pycnidiospore, 4. Uniseptate pycnidiospore, 5. Macrotype non-septate pycnidiospore, 6. Microtype non-septate pycnidiospore, 7. Whitish masses of exuded ascospore formed on the ostiole of perithecia, 8. Asci and ascospore.

blotter test. Isolate no. 6 and 8 obtained from pumpkin were produced numerous pycnidia on WALM, but perithecia sparsely near to inoculated portion of substrautm. Isolates (no. 1 and 2) obtained from cucumber produced numerous perithecia, but pycnidial formation were rather lower than others.

On seed the first evidence of the pathogen was the early appearance of pycnidia either on the seed coat or radicle. The pycnidia have been found three days after inoculation. At first partially submerged pycnidia were brownish and turned dark brown later. Dull pinkish oozing spore masses formed drops from the pycnidia. The pycnidia were nearly spherical, slightly longer than wide and somewhat flattened at the apex, where the conidia were released through a single pore (Fig. 2). They varied greatly in size

Table II. Types of sporulating structures of four isolates of *Didymella bryoniae* from cucumber and pumpkin seeds on different media at 20°C under NUV.

Isolate no.	PDA	V-8 juice agar	WALM	Seed
1 (cucumber)	(pycnidia) & empty black bodies	perithecia & (pycnidia)	perithecia & (pycnidia)	pycnidia
2 (cucumber)	perithecia & (pycnidia)	perithecia & (pycnidia)	perithecia	pycnidia & (perithecia)
6 (pumpkin)	pycnidia	(perithecia) & pycnidia	(perithecia) & pycnidia	pycnidia
8 (pumpkin)	pycnidia	(perithecia) & pycnidia	(perithecia) & pycnidia	(perithecia) & pycnidia

Table III. Sporulating characters of pycnidiospores of four isolates of *Didymella bryoniae* from cucumber and pumpkin seeds on different media at 20°C under NUV.

Isolate no.	PDA	V-8 juice agar	WALM	Seed
1 (cucumber)	unicellular spores	unicellular, uniseptate & (biseptate spores)	unicellular, uniseptate & (biseptate spores)	unicellular, uniseptate & (biseptate spores)
2 (cucumber)	unicellular spores	unicellular & (uniseptate spores)	no	unicellular, uniseptate & (biseptate spores)
6 (pumpkin)	unicellular spores	unicellular & (uniseptate spores)	unicellular & (uniseptate spores)	unicellular & (uniseptate spores)
8 (pumpkin)	unicellular spores	unicellular & (uniseptate spores)	unicellular & (uniseptate spores)	unicellular & (uniseptate spores)

Table IV. Types of pycnidiospores of *Didymella bryoniae* produced on seed and culture media at 20°C under near ultraviolet light.

Substrate	Type of pycnidiospore	Size of pycnidiospore
Cucurbit seed	microtype(non-septate)	3−11×1.5−3μm
	macrotype(non-septate, uniseptate & biseptate)	8−13×3.5−4μm
WALM	macrotype(non-septate & uniseptate)	8.5−10.5×2.6−3.0μm
V-8 juice Agar	macrotype(non-septate & uniseptate)	8.0−12.0×3.0−6.0μm
PDA	microtype(non-septate)	5.0−7.5×1.6−2.5μm

Table V. Description of perithecial structure of *Didymella bryoniae* on seed and WALM.

Fruiting structure	Description
Ascospore	hyaline, 2-celled, ellipsoid, ends mostly rounded, slightly constricted at the septum, guttulate, 9.0−15.5×4.5−9.0μm
Ascus	cylinderical to subclavate, short stipitate or sessile, 8 spored, ascus wall bitunicate, 31−75×6−12μm
Perithecium	globose,to slightly elongated,immersed, becoming erumpent, black, opening by apical papillate ostiole and whitish spore masses formed on the ostiole 62∼297 diam. μm
Pseudoparaphyses	hyaline, septate and branched

within a usual range in diameter of 45μ to 181μ. Measurements of pycnidiospores on seed and culture media are given in Table Ⅳ.

Perithecia were occasionally observed on seeds of cucumber and pumpkin. They usually developed later than pycnidia, but on pumpkin seeds, perithecia have been found three days after inoculation. Morphological characters of perithecium of this fungus on seed are given in Table Ⅴ and Fig. 7.

Most of the pycnidiospores obtained from each isolate of this fungus grown on PDA were non-septate and microtype (Fig. 6). They contained occasionally two oil globules. Pycnidiospores of this fungus when grown on V-8 juice agar were quite distinctive from those on PDA (Tables Ⅲ&Ⅳ). Non-septate pycnidiospores were predominanted in all isolates, but macrotype of the non-septate (Fig. 5) and a number of uniseptate pycnidiospores (Fig. 4) were produced. Pycnidiospores produced on V-8 juice agar were similar to those produced on radicle of seeds. Pycnidiospores of each isolate of *D. bryoniae* which grown on WALM were quite distinctive from those grown on PDA, but very similar to those grown on V-8 juice agar (Tables Ⅲ&Ⅳ). Macrotype of non septate (Fig. 5) and uniseptate of pycnidiospores (Fig. 4) were produced. These also closely similar in shape and size with those produced on V-8 juice agar and radicle of cucurbit seeds. On seed the pycnidiospores were hyaline, shortly cylindrical with rounded ends, mostly non-septate (Fig. 6), but rarely uniseptate ones were also found. On radicle of cucumber seed, the pycnidiospores were non-septate (Fig. 5) and uniseptate, but small percentage biseptate (Fig. 3) with somewhat constricted at septa. The spores seem to varied considerably in size and shape, depending on the seed samples.

Morphological characters of ascus, ascospore and pseudoparaphyses are given in Table V and Fig. 8. Both asci and ascospores on WALM were identical in size and shape with those found on cucurbit seeds.

Discussion

The cultural characters of the four isolates of *D. bryoniae* grown on Difco potato dextrose agar, V-8 juice agar and water agar leaf meium were studied and compared with those on seed. It was found that variability in cultural characters existed depending on isolates and media. Chiu & Walker(1949) reported that pycnidia bearing either micropycnidiospores or macropycnidiospores were present in the sporulating cultures of this fungus and production of one or both types on certain media varied with the strain of the organism. On PDA, most of the isolates used in this study produced non-septate microtype of pycnidiospores. The imperfect stage of *D. bryoniae* when producing non-septate conidia on culture media might readily be mistaken for other fungi, as done on seed.

Production of macrotype of non-septate and uniseptate of pycnidiospores, and the sexual stage was extremely important in the identification of this pathogen. On V-8 juice agar and water agar leaf medium, macrotype of non-septate and uniseptate pycnidiospores were produced. These were quite distinctive from those grown on PDA but closely similar in shape and size with those produced on radicle of cucurbit seeds.

Moreover all isolates produced perfect stage of this fungus on V-8 juice agar and WALM under near ultravilolet light, but some isolates produced perithecia sparsely on both media. Curren(1969) found that V-8 juice agar was the only medium in which there was abundant production of perithecia of this fungus. Perithecia were identical in appearance on both media to compared with those found on cucumber and pumpkin seeds. Although some variation in the size of spores and fruiting bodies was noted between the measurements of the pathogen grown on seed and WALM and those reported for this fungus by Hemmi (1922), Waint(1945) and Chiu & Walker (1949), the differences were not great. Therefore, despite the difficulty of classifying the imperfect stage of this fungus on seed and PDA culture, it was possible to identify all the isolates of *Didymella bryoniae* based on the production of perfect stage. The early appearance of *D. bryoniae* on seed was not easy to identify.

There was much confusion with saprophytic pycn-

idia forming fungi such as *Phoma*. However in later stages the pathogen produced typical symptoms on radicle with numerous pycnidia. Most of the pycnidiospores in these pycnidia were non-septate, some uniseptate and few biseptate compare to slightly smaller, generally non-septate spores in pycnidia produced on seed coat. Experience gained in examining infection of this fungus in nine seed samples indicate that percentage of infection of *D. bryoniae* should be recorded between 7~10 days after inoculalion. Pleomorphism and variability of the imperfect stage of this fungus has been responsible for some of the confusion in the identification work in this study. For this reason, many previous workers have discussed extensively the taxonomic position of this pathogen(Hemmi 1922: Waint 1945: Chiu & Walker 1949).

Perithecia of *Didymella bryoniae* were observed on the seed coat of cucumber and pumpkin, but never on radicle. The appearance of perithecia was quite distinctive from pycnidia. It could be an easy a way to detect this fungus on seed. However, the perithecial formation varied greatly in different samples, no perithecia were seen in the six other infected samples.

摘 要

自然狀態에서 *Didymella bryoniae*에 罹病된 오이 및 호박 種子를 濕紙法으로 處理하여 不完全 世代와 完全 世代의 特徵을 調査하고 Difco PDA, V-8쥬스 寒天 및 water agar leaf medium에서의 特徵과 比較 하였다.

병든 種子가 發芽할 때 種皮나 胚軸에 形成된 *Didymella bryoniae*의 柄胞子와 Difco PDA, V-8 juice 寒天 및 WALM에 形成된 本菌의 柄胞子는 形態的으로 많은 差異가 나타난다. 즉 PDA에 形成되는 柄胞子는 無色 小型單胞子가 대부분이며, V-8 juice寒天 및 WALM에 形成되는 것은 大型單胞子外에 隔膜이 하나 있는 胞子를 형성하고 또 菌株에 따라서는 隔膜이 두개 있는 胞子를 형성하므로써 많치 병든 種皮나 胚軸에 形成된 것과 흡사하였다.

한편 子囊殼은 병든 種子의 標本에 따라 잘 形成되는 것도 있었으나 大部分의 標本에서는 形成되지 않았다. 外部形態的인 特徵은 색갈이 진한 黑色이며 乳頭狀殼孔 위에 白色의 胞子塊가 形成되는 特徵을 가지고 있어서 쉽게 柄子殼과 區別할 수 있었다. 不完全 世代인 柄子殼이나 柄胞子와 같이 子囊殼의 殼房과 胞子의 크기에는 큰 變異가 없었다.

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〈Received February 2, 1982〉